

This product is for research use only (not for diagnostic or therapeutic use)

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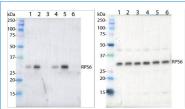
# Product no AS19 4291 Anti-RPS6A-P237 | Phosphorylated (Ser237) 40S ribosomal protein S6-1

### **Product information**

| Immunogen      | KLH-conjugated peptide derived from Arabidopsis thaliana RPS6A with phosphorylated Ser237, UniProt: <u>048549</u> , TAIR: <u>At4g31700</u>   |
|----------------|--|
| Host           | Rabbit   |
| Clonality      | Polyclonal   |
| Purity         | Immunogen affinity purified serum in PBS pH 7.4.   |
| Format         | Lyophilized  |
| Quantity       | 50 μg  |
| Reconstitution | For reconstitution add 50 $\mu$ l, of sterile water  |
| Storage        | Store lyophilized/reconstituted at -20 °C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube. |

### **Application information**

| Recommended dilution      | 1 : 1000 (WB)  |
|---------------------------|--|
| Expected   apparent<br>MW | 28.3 kDa   |
| Confirmed reactivity      | Arabidopsis thaliana   |
| Predicted reactivity      | Actinidia rufa, Ananas comosus, Beta vulgaris, Cajanus cajan, Capsicum chinense, Citrus clementina, Gossypium<br>australe, Olea europaea subsp. europaea, Oryza sativa, Panicum miliaceum, Populus alba, Sesamum indicum, Senna<br>tora, Solanum lycopersicum, Vigna unguiculata<br>Species of your interest not listed? <u>Contact us</u> |
| Not reactive in           | No confirmed exceptions from predicted reactivity are currently known  |
| Selected references       | To be added when available, antibody released in November 2020.  |



Left panel is developed with anti-RPS6-Ser237, while right panel anti-RPS6, which serves as a loading control.

#### Lanes:

1 and 4 - 0.5 µg of Arabidopsis thaliana whole leaf extract from untreated leaf discs

2 and 5 - 0.5 µg of Arabidopsis thaliana whole leaf extract from leaf discs treated with 10 mM glutamine (TOR activator) for 8 hours

3 and 6 - 0.5 µg of Arabidopsis thaliana whole leaf extract from leaf discs treated with 2 µM AZD-8055 (TOR inhibitor) for 8 hours

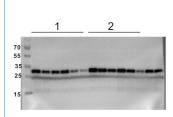
0.5 µg/well of total protein extracted freshly from mature *Arabidopsis thaliana* leaves with 50 mM HEPES pH 7.5, 5mM NaF, 2.5 mM NaPPi, 25 mM B-phosphoglycerol, Roche Complete inhibitor tablet (1x), 2% PVPP, 2 mM PMSF) and denatured with 2x SDS sample buffer (80 mM tris pH 6.8, 2% SDS, 10% glycerol, 100 mM DTT, bromophenol blue) at 99 °C for 3 min. were separated on AnyKd (BioRAD) gradient % SDS-PAGE and blotted 1h to PVDF (pore size of 0.45 um) using semi-dry transfer. Blot was blocked with 2% milk for 1h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1 000 in TBS-T + 2% milk powder ON/4 °C with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed 3 times for 5 min in TBS-T at RT with agitation. Blot was incubated in Agrisera matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, <u>AS09 602</u>) diluted to 1:25 000 in TBS-T + 2 % milk powder for 1h/RT with agitation. The blot was washed as above and developed for 5 min with Agrisera ECLBright with GE Amersham Imager 600. Exposure time was ~60.seconds.

Courtesy of Dr. Brendan O'Leary, University of Western Australia, School of Molecular Sciences, ARC Centre for Plant Energy Biology, Australia

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20 µg/well of total protein freshly extracted freshly from 16 d old *Arabidopsis thaliana* seedlings total leaf of wildtype **(1)** and mutant **(2)** with 2x SDS loading dye exact buffer and denatured at 80 °C for 5 min.were separated on 12% SDS-PAGE and blotted 10 min to PVDF (pore size of 0.2 um), using using Turbo transfer (BioRad). Blot was blocked with 5% milk for 1h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1 000 in TBS-T ON/4 °C with agitation. The antibody solution was decanted and the blot was rinsed briefly once, then washed 5 times for 10 min in TBS-T at RT with agitation. Blot was incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated) diluted to 1:15 000 in for 1h/RT with agitation. The blot was washed as above and reaction was visualized with chemiluminescence, following manufacture's instructions. Exposure time was 5 seconds.

Courtesy of Dr. Arsheed Sheikh, King Abdullah University of Science and Technology (KAUST), Saudi Arabia

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